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A new freshwater crab of the genus *Geothelphusa* Stimpson, 1858 (Crustacea: Decapoda: Brachyura: Potamidae) from Yakushima Island, southern Kyushu, Japan

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Abstract.—A new freshwater crab, *Geothelphusa marmorata,* is described from Yakushima Island of Kagoshima Prefecture, southern Kyushu, Japan. It is differentiated from congeners by possession of distally narrowed eyes, chocolate brown or dark red carapace with scattered black speckles in life, stout gastric cristae, a deep H-shaped median gastro-cardiac depression, and laterally curved penultimate segment of the male first gonopod with a mesially curved ultimate segment. This is the second species of *Geothelphusa* Stimpson known from Yakushima Island, and the twelfth species from Japan. Electrophoretic analysis of 15 gene loci suggests that *G. marmorata,* new species, *G. exigua* Suzuki & Tsuda and *G. dehaani* (White) are reproductively isolated.

Freshwater crabs of the genus *Geothelphusa* Stimpson, 1858 are distributed from Taiwan, through the Ryukyu Islands, to the Japanese mainland. Recently, a large number of species were reported from Taiwan and Ryukyu Islands, and 39 species are now recognized in the genus (Shy et al. 1994, Shy & Ng 1998, Tan & Liu 1998). In Japan, 11 species are currently known, i.e., *G. dehaani* (White, 1847), *G. obtusipes* Stimpson, 1858, *G. sakamotoana* (Rathbun 1905), *G. aramotoi* Minei, 1973, *G. tenuimana* (Miyake & Minei 1965), *G. levicervix* (Rathbun 1898), *G. candidiensis* Bott, 1970, *G. miyazakii* (Miyake & Chiu 1965), *G. exigua* Suzuki & Tsuda, 1994, *G. shokitai* Shy & Ng, 1998, and *G. minei* Shy & Ng, 1998 (see also de Haan 1835, Rathbun 1904, Bott 1967, Minei 1974b, Shy et al. 1994). Of these, *G. dehaani* is widely distributed on the Japanese mainland (north of Honshu southward to Nakano-shima of the Tokara Islands, south of Kyushu). The other 10 species are restricted to the southern Kyushu or the Ryukyu Islands, including Amami-ohshima.

During our current study of the population genetics and geographic distribution of *G. dehaani* and *G. exigua* in southern Kyushu, unusual specimens of *Geothelphusa* species were found on Yakushima Island in Kagoshima Prefecture. The unusual eyes, coloration of body, and structure of the male first gonopods of these crabs indicate that they represent a new species that is herein described and illustrated. In addition, a genetic analysis based on electrophoresis is included.

Materials and Methods

For electrophoretic analysis, a total of 201 specimens of *G. dehaani* were collected from three populations (174 specimens from Kotsuki River of the Kagoshima mainland, 20 from Hitotsutani River of the Kagoshima mainland, and seven from Anboh River of the Yakushima Island); 40 specimens of *G. exigua* were collected from two populations (20 specimens from Hitotsutani River and 20 from Kamiharai River of the Kagoshima mainland); and 17 specimens of the new species were collected

Table 1.—List of enzymes and protein, and buffer systems used in electrophoretic analysis. CAPM 6.0; Citric acid-aminopropyl morpholine, pH 6.0: CAPM 7.0; Citric acid-aminopropyl morpholine, pH 7.0: and CT 8.0; Tris-citric acid, pH 8.0.

Enzyme and protein (Abbreviation and E. C. number)	Symbol for locus	Buffer system
Aspartate aminotransferase (AAT, 2.6.1.1)	*AAT**	CAPM 6.0
Adenylate kinase (AK, 2.7.4.3)	*AK**	CAPM 7.0
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.2.1.12)	*GAPDH**	CAPM 6.0
Glucose-6-phosphate isomerase (GPI, 5.3.1.9)	*GPI**	CAPM 6.0, CAPM 7.0
Hexokinase (HK, 2.7.1.1)	*HK**	CT 8.0
Isocitrate dehydrogenase (IDHP, 1.1.1.42)	*IDHP-1**	CAPM 7.0
	*IDHP-2**	CAPM 7.0
Lactate dehydrogenase (LDH, 1.1.1.27)	*LDH**	CT 8.0
Malate dehydrogenase (MDH, 1.1.1.37)	*MDH-1**	CAPM 7.0
	*MDH-2**	CAPM 7.0
Malic enzyme (ME, 1.1.1.38)	*ME**	CT 8.0
Mannose-6-phosphate isomerase (MPI, 5.3.1.8)	*MPI**	CT 8.0
Phosphogluconate dehydrogenase (PGDH, 1.1.1.44)	*PGDH**	CAPM 7.0
Phosphoglucomutase (PGM, 5.4.2.2)	*PGM**	CT 8.0
General protein (PROT)	*PROT**	CAPM 7.0

from the Anboh River during 1997. Specimens used for electrophoretic analysis were stored at −35°C with a small volume of freshwater. Muscles were extracted and homogenized with an equal volume of cold distilled water. Horizontal starch gel electrophoresis was performed for the detection of enzyme and protein variations (Table 1). Locus and gene nomenclature follows that of Shaklee et al. (1990). Multiple loci for a given enzyme were distinguished by numerals, with "*−1**" representing the most anodally-migrating isozyme. All alleles studied are designated alphabetically. The genetic distance was calculated using Nei's formula (Nei 1972). All the specimens were collected by the junior author.

The holotype and a paratype are deposited in the Kitakyushu Museum of Natural History, Kitakyushu (KMNH), and additional paratypes in the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM), and Marine Biological Laboratory, Faculty of Fisheries, Kagoshima University, Kagoshima (KUMB). Measurements shown in parentheses under "Material examined" indicate the maximum carapace width in millimeters. Abbreviations used include: M, male; F, female.

Family Potamidae Ortmann, 1896
Genus *Geothelphusa* Stimpson, 1858
Geothelphusa marmorata, new species
Figs. 1–3, Tables 1–3

Material examined.—River Anboh: Arakawa, 1280 m alt., 24 Oct 1998: holotype, M (29.4), KMNH-IvR 900005, paratype, F (21.1), KMNH-IvR 900006, M (23.2), F (28.9), USNM 268571, 1120 m alt., 8 Jul 1997; 3 M (30.5, 26.8, 21.8), KUMBcr 1053 (used for the electrophoretic analysis).

Diagnosis.—Penultimate segment of male first gonopod slightly curved laterally, ultimate segment strongly curved mesially, cone-shaped, with terminal aperture. Ocular peduncle swollen proximally, cornea small. Gastric cristae stout, H-shaped median gastro-cardiac depression distinct. In life, carapace and pereopods chocolate brown or dark red with scattered black speckles.

Description.—Carapace much broader than long, smooth, devoid of setae (Fig. 1a); faint, short oblique striae on epibranchial and posterolateral regions; epi- and uro-gastric regions distinct, former divided into 2 stout gastric cristae by deep median groove; H-shaped median gastro-cardiac depression deep, wide; deep transverse

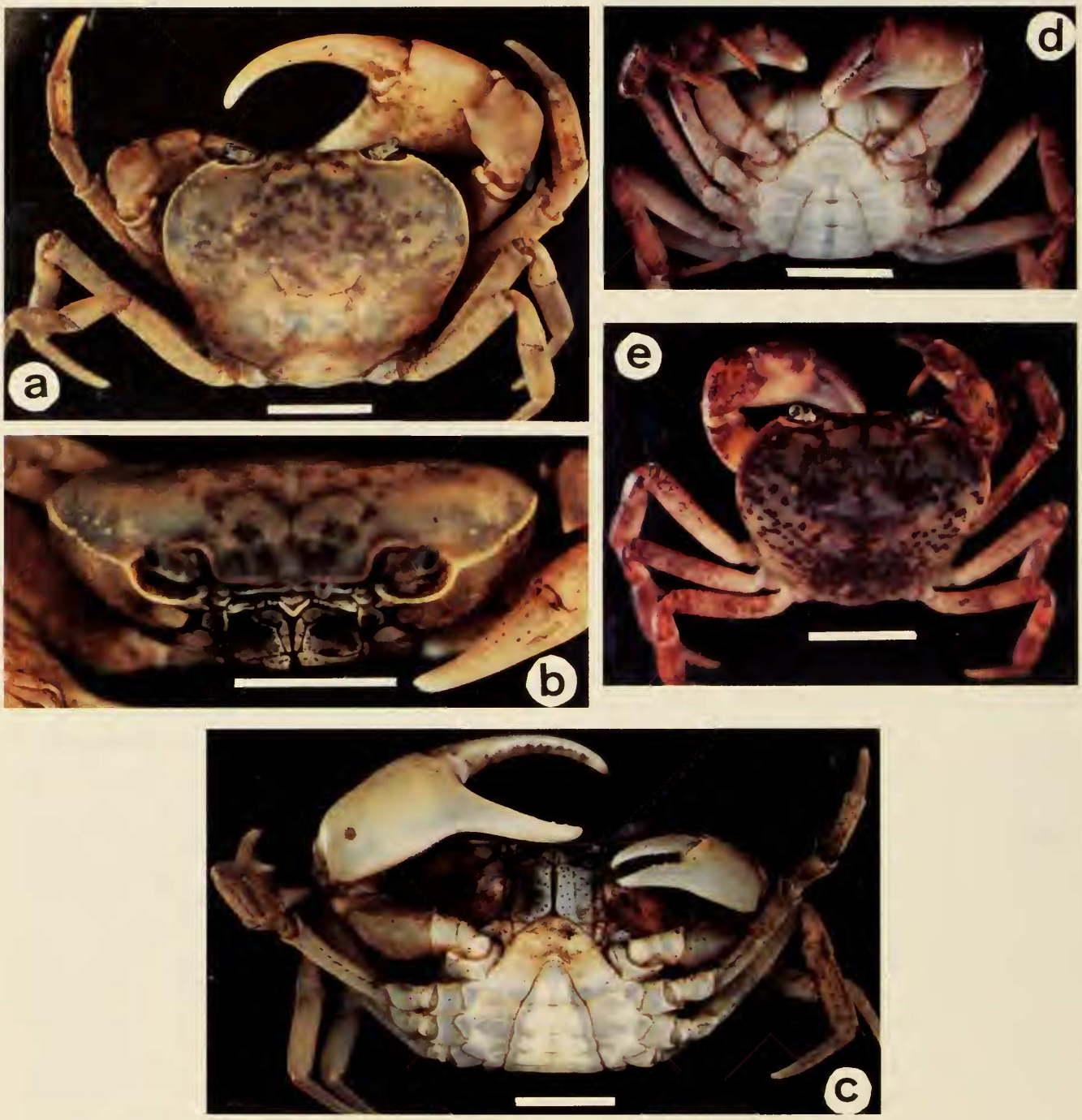

Fig. 1. *Geothelphusa marmorata,* new species, male holotype (KMNH-IvR 900005): a, dorsal view; b, frontal view; c, ventral view. Male paratype (USNM 268571): d, ventral view; e, dorsal view. Scales indicate 10 mm.

groove between cardiac and intestinal regions; cervical groove obsolete on epibranchial region. Anterolateral margin of carapace cristate, lined with fine rounded granules; epibranchial notch rudimentary. Posterior margin of epistome divided into 3 parts by 2 deep notches (Fig. 1b), granules present on lower edge of epistome, absent medially. Lower orbital margin and groove between subhepatic and pterygostomian regions lined with faint granules.

Eyestalk short, proximally swollen, distally slender. Cornea small, slightly wider than distal portion of ocular peduncle (Fig. 1a, b).

Merus of third maxilliped broad, squarish, with deep depression (Fig. 1b). Palp 3-segmented, connected on inner distal angle of merus, tip of palp not below distal margin of ischium. Exopod slender, longer than ischium, with small 5-segmented flagellum (Figs. 1c, d, 2a; exopodal flagellum damaged in holotype).

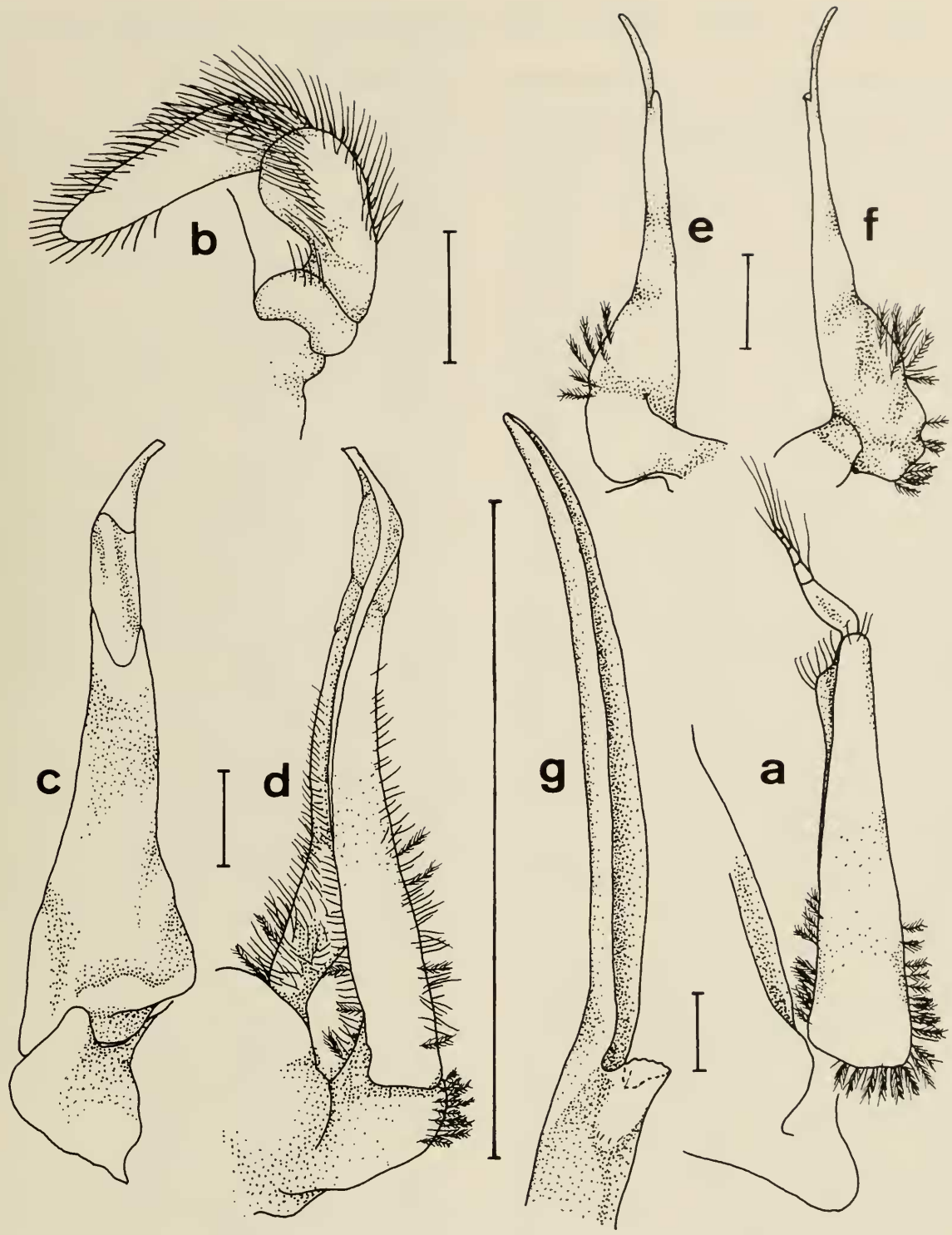

Fig. 2. *Geothelphusa marmorata,* new species, male paratype (USNM 268571): a, exopod of third maxilliped, frontal view; b–g, male holotype (KMNH-IvR 900005): b, left mandibular palp, ventral view; c, left first gonopod, dorsal view; d, same, ventral view; e, left second gonopod, dorsal view; f, same, ventral view; g. tip of left second gonopod, dorsal view. Scales indicate 1 mm.

Chelipeds asymmetrical in males, symmetrical in females; movable finger of large cheliped strongly curved in large male (not so in small male) (Fig. 1a, c–e); palm smooth, outer surfaces convex (Fig. 1a, c, d). Carpus of large cheliped almost smooth, with stout inner tooth, below which is a low projection (Fig. 1a, e). Carpus of small cheliped without any projection below stout inner tooth.

Palp of mandible 3-segmented (Fig. 2b); distal segment uniramous, sickle-shaped; median segment longer than wide, distal

half expanded; proximal segment short, stout.

Penultimate segment of adult male first gonopod (G 1) gently curved laterally (Fig. 2c, d), synovial membrane short, about 3 times as long as broad (Fig. 2c), ultimate segment strongly curved mesially (Fig. 2c, d), cone-shaped, with terminal aperture. Male second gonopod (G 2) slender, flat, weakly convex at proximal part, a small cup-like structure on distal one-fourth, tip of G 2 concave (Fig. 2e, f, g). G 2 shorter than G 1, tip of G 2 not protruding from aperture of G 1 when coupled.

Color in life.—Carapace and pereopods (Fig. 1a, e) chocolate brown or dark red with scattered black speckles. Lower part of palm and inmovable finger of both chelipeds white in large male, movable finger and upper part of palm of chelipeds chocolate brown. In small males and females, lower part of palm of both chelipeds white, fingers and upper part of palm dark red. Otherwise, no color variation observed between sexes and sizes.

Genetic characteristics.—Among fifteen gene loci coding for twelve enzymes and one protein, allelic substitution was observed between *G. marmorata,* new species, and *G. exigua* at Lactate dehydrogenase (*LDH**), Isocitrate dehydrogenase-1 (*IDHP-1**), and Phosphogluconate dehydrogenase (*PGDH**) loci (Table 2), and between *G. marmorata* and *G. dehaani* at *PGDH** locus. Nei's genetic distances (*D*) were calculated between all samples based on the fifteen loci (Table 3). The *D* values of *G. dehaani* populations and *G. exigua* populations were low (0.016–0.066 and 0.037, respectively). However, the *D* values between *G. marmorata* and *G. dehaani* were high, ranging from 0.155 to 0.2, and the values between *G. marmorata* and *G. exigua* were higher (0.473 and 0.534).

Etymology.—The specific name is derived from the Latin *marmoratus,* marbled, alluding to the characteristic color pattern of the new species.

Remarks.—The saber-like G 1 and the 3-segmented mandibular palp with uniramous distal segment present in the new species are characteristics of the genus *Geothelphusa* (see Bott 1970). The medium-sized carapace, anterolateral margin lined with small rounded granules and absence of an epibranchial tooth ally *G. marmorata* with *G. dehaani, G. exigua, G. bicolor, G. miyazakii, G. candidiensis, G. ferruginea, G. tali, G. shokitai,* and *G. minei,* from which it is distinguished by several features.

The life color of the carapace with scattered black speckles easily distinguishes *G. marmorata* from *G. dehaani, G. bicolor, G. miyazakii, G. ferruginea,* and *G. tali.* The most definitive differences are in the structure of the G 1. The G 1 ultimate segment in eight related species, except for *G. exigua,* is straight or slightly curved mesially (Bott 1967, 1970; Minei 1973, 1974a; Suzuki & Tsuda 1994, Shy et al. 1994, Shy & Ng 1998). The ultimate segment in *G. exigua* is curved laterally and tapering, and has a subterminal aperture. However, in *G. marmorata,* this segment is strongly curved mesially, ending in a papilla-like tip with a terminal aperture. The eyestalks in the eight related species are constricted medially, and the cornea and proximal part of the ocular peduncle are swollen. In *G. marmorata,* only the proximal part of the ocular peduncle is swollen, while the cornea and the distal part of the peduncle are proportionately narrower as in *G. exigua.*

Previous genetic studies revealed that the different populations of *G. dehaani* in Japan exhibit varying allele frequencies in some gene loci in Japan (Sugawara & Gamo 1984, Nakajima & Masuda 1985, Aotsuka et al. 1995, Ikeda et al. 1998). If allelic substitution is observed at any gene locus among the different morphological groups based on coloration, shape of G 1, and so on, there is a possibility of the groups actually representing different species. In the previous studies, there was no allelic substitution in any gene locus among the different morphological groups. However, Ikeda et al. (1998) observed an allelic replace-

Table 2.—Allele frequencies at 15 loci for 3 populations of *G. dehaani*, 2 populations of *G. exigua* and 1 population of *G. marmorata* in Kagoshima Prefecture.

Locus	Allele	*G. dehaani*			*G. exigua*		*G. marmorata*
		Koutsuki	Hitotsutani	Anboh	Hitotsutani	Kamiharai	Anboh
*AAT**	**a*	0.027	0.025	0.000	0.975	1.000	0.059
	**b*	0.922	0.975	0.929	0.025	0.000	0.941
	**c*	0.051	0.000	0.071	0.000	0.000	0.000
*AK**	**a*	0.961	1.000	1.000	1.000	0.750	1.000
	**b*	0.028	0.000	0.000	0.000	0.250	0.000
	**c*	0.011	0.000	0.000	0.000	0.000	0.000
*GAPDH**	**a*	1.000	1.000	1.000	1.000	1.000	1.000
*GPI**	**a*	0.253	0.025	0.000	0.600	0.200	0.059
	**b*	0.726	0.475	0.000	0.000	0.275	0.647
	**c*	0.018	0.050	0.000	0.400	0.525	0.000
	**d*	0.003	0.425	1.000	0.000	0.000	0.235
	**e*	0.000	0.025	0.000	0.000	0.000	0.059
*HK**	**a*	0.085	0.000	0.000	0.000	0.000	0.000
	**b*	0.755	1.000	0.786	0.917	0.975	0.735
	**c*	0.160	0.000	0.214	0.083	0.025	0.265
*IDHP-1**	**a*	0.891	1.000	1.000	0.000	0.000	1.000
	**b*	0.070	0.000	0.000	0.000	0.000	0.000
	**c*	0.039	0.000	0.000	0.000	0.000	0.000
	**d*	0.000	0.000	0.000	1.000	1.000	0.000
*IDHP-2**	**a*	1.000	1.000	1.000	0.050	0.000	1.000
	**b*	0.000	0.000	0.000	0.950	1.000	0.000
*LDH**	**a*	1.000	1.000	1.000	0.000	0.000	1.000
	**b*	0.000	0.000	0.000	1.000	1.000	0.000
*MDH-1**	**a*	0.964	0.900	1.000	0.975	0.950	1.000
	**b*	0.036	0.100	0.000	0.025	0.050	0.000
*MDH-2**	**a*	0.994	0.975	1.000	1.000	1.000	1.000
	**b*	0.006	0.025	0.000	0.000	0.000	0.000
	**c*	0.000	0.000	0.000	0.000	0.000	0.000
*ME**	**a*	1.000	1.000	1.000	1.000	1.000	1.000
*MPI**	**a*	1.000	1.000	1.000	1.000	1.000	1.000
*PGDH**	**a*	0.000	0.000	0.000	0.000	0.000	1.000
	**b*	0.991	1.000	1.000	1.000	1.000	0.000
	**c*	0.009	0.000	0.000	0.000	0.000	0.000
*PGM**	**a*	0.875	0.925	0.571	0.925	0.375	0.971
	**b*	0.018	0.075	0.214	0.075	0.625	0.000
	**c*	0.108	0.000	0.214	0.000	0.000	0.029
*PROT**	**a*	1.000	1.000	1.000	0.000	0.000	0.059
	**b*	0.000	0.000	0.000	1.000	1.000	0.941

Table 3.—Genetic distance among 3 populations of *G. dehaani*, 2 populations of *G. exigua* and 1 population of *G. marmorata* in Kagoshima Prefecture.

		G. dehaani			*G. exigua*	
		Koutsuki (I)	Hitotsutani (II)	Anboh (III)	Hitotsutani (IV)	Kamiharai (V)
G. dehaani	(II)	0.016				
	(III)	0.032	0.066			
G. exigua	(IV)	0.469	0.462	0.519		
	(V)	0.520	0.518	0.559	0.037	
G. marmorata	Anboh (VI)	0.156	0.155	0.200	0.473	0.534

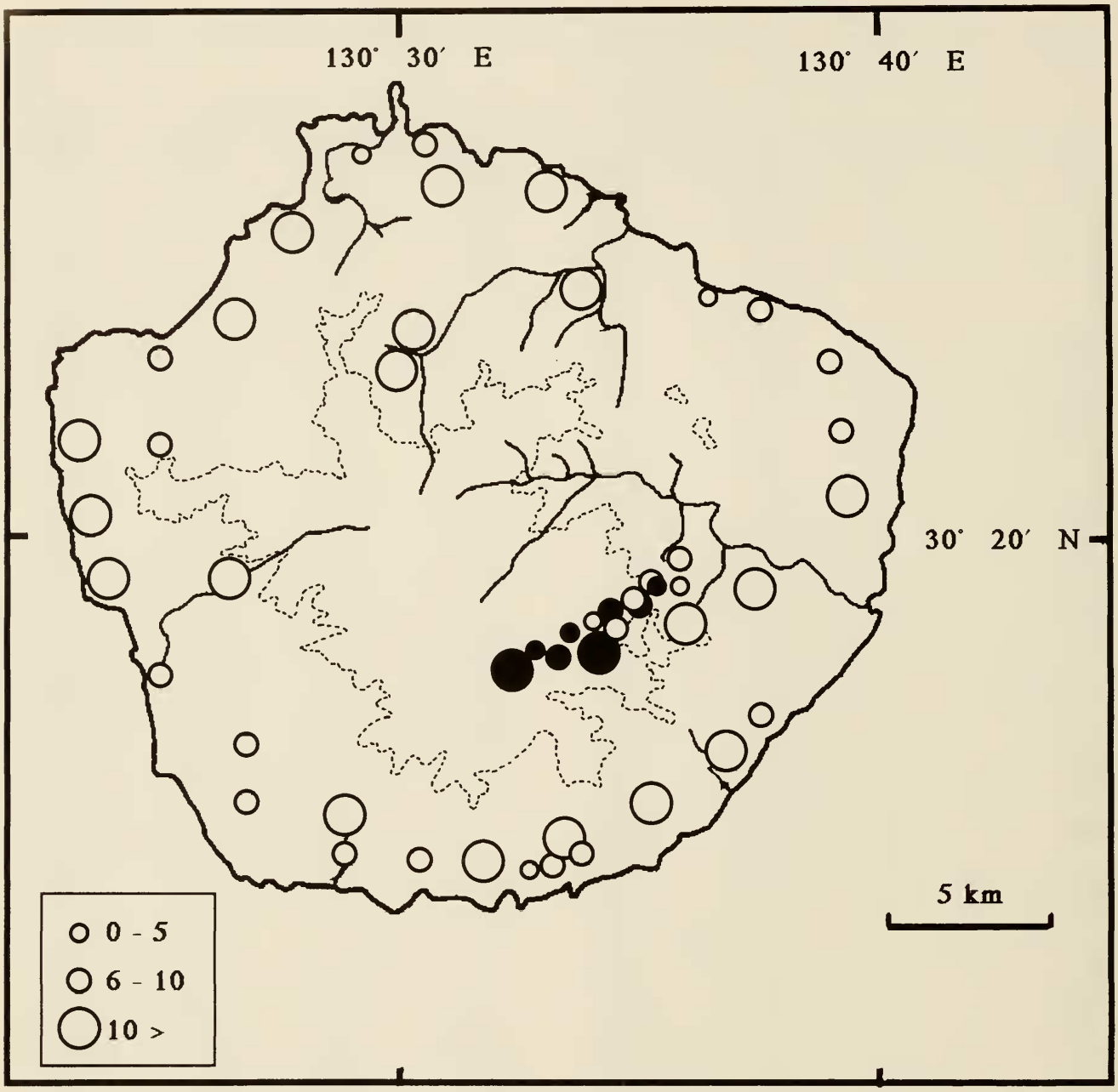

Fig. 3. Distribution and abundance of *G. marmorata,* new species (solid circle) and *G. dehaani* (White, 1847) (white circle) in Yakushima Island, Kagoshima, Japan. Arabic numerals correspond to size of circles in the square at bottom left, and indicate the number of crabs captured per ten minutes by one person. Broken line shows 1000 m contour line.

ment at three loci between color morphs representing allopatric populations. No morphological differences in G 1 structure have been reported among those populations thus far. In this study, allelic substitution was observed at three loci among *G. marmorata, G. dehaani,* and *G. exigua.* In addition, *G. marmorata,* and *G. dehaani* are sympatric in Anboh River, Yakushima Island. The observed allelic substitutions, therefore, strongly indicate the presence of reproductive isolation among three species. The genetic distance (*D* values) further suggest that *G. marmorata,* belongs to a different evolutionary lineage from the Anboh population of *G. dehaani.*

Distribution.—The specimens of *G. marmorata,* examined have been obtained only in the area above 950 m altitude on Yakushima Island, Kagoshima Prefecture (Fig. 3). *Geothelphusa marmorata,* and *G. dehaani* are sympatric, having been taken to-

gether at some locations from 950 m to 1350 m altitude.

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